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Decomposition and Soil Organic Matter Dynamics

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Introduction

Decomposition is the process whereby dead organic material is broken down to its constituent parts, ultimately to carbon dioxide (CO_2) and inorganic ions such as ammonium (NH_4^+), calcium (Ca^{2+}), potassium (K^+), and other elements originally assimilated by the organisms now decomposing. At various points along this path the decomposing organic matter is consumed and metabolized by other organisms, which themselves will die and decompose. Decomposition is thus a dynamic recursive process that involves a complex array of physical, chemical, and biological interactions that complete the biogeochemical nutrient cycles.

Rates of decomposition vary tremendously as a function of the structure and chemical composition of the organic matter being decomposed (substrate quality), the abiotic environment in which decomposition is occurring (temperature, moisture, and aeration status in particular), and the degree to which the decomposing substrate is exposed to heterotrophs, both microbial and faunal (Kurcheva 1960; Bunnell et al. 1977; Jenny et al. 1949; Swift et al. 1979; Paustian et al. 1997). One might thus expect differences in rates of decomposition both within and between ecosystems. Within-ecosystem variability will occur as different materials are deposited and decompose in microhabitats that range from plant canopies to different positions along topographic gradients, different positions within soil profiles, and even different positions within soil aggregates. Differences among ecosystems arise from expressions of environmental and biotic gradients at larger scales, especially with respect to climate, ecosystem fertility, and successional status (Olson 1963; Meentemeyer 1978; Melillo et al. 1982; Wallace and Freedman 1986; Aber et al. 1990).

Operationally, in terrestrial ecosystems, decomposition typically defines the breakdown of organic matter into either inorganic ions or into an organic matter size class that permits its entry into the soil organic matter (SOM) pool. The SOM pool receives the breakdown products of decomposition, and the further decomposition and humification of these products falls under the general rubric of SOM dynamics—which also includes the turnover of microbes and soil invertebrates that further oxidize and assimilate SOM.

Typically, decomposition is measured as mass and nutrient loss from dead organic material placed in that part of the ecosystem in which it normally occurs—usually the soil surface for senescent plant leaves, branches, stems, and other aboveground litter, and various soil horizons for roots, fungi, and other belowground organisms or parts thereof. More often than not, the usual strategy for assessing mass loss is to entrain a known quantity of material at a specific location and to then periodically evaluate it or a subsample for mass and nutrient loss.

Methods for assessing organic matter dynamics (as opposed to decomposition per se) are varied and often more difficult to interpret than decomposition assays owing to the heterogeneous nature of SOM. The most comprehensive and least controversial measure is that of the total SOM pool itself. Combustion techniques provide an adequate representation of total organic carbon (C) stores in soil and can be used to document changes in these stores

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over decadal to century intervals. These measures are particularly important for assessing changes in soil C pools as a function of land use change (e.g., Haas 1957) and as a function of changes in potential feedbacks in the global C budgets in response to climate change (e.g., Schlesinger 1990; IPCC 1996). Combustion techniques are less useful for understanding the role of SOM in ecosystem C dynamics because combustion techniques do not adequately measure C availability.

The availability of C to heterotrophs differs markedly from one ecosystem to another and can be independent of total C stores. For example, ecosystems with identical soil C stores may have very different soil C cycles because of marked differences in the availability of C stores to the heterotrophic community. Soil C takes a wide variety of organic forms, ranging from simple sugars to humified substances with large molecular weights and complex aromatic structures. These latter compounds are highly resistant to microbial attack, especially when protected by association with stable soil aggregates and clay minerals, and may thus have turnover times on the order of millennia.

Efforts to characterize SOM in ecologically meaningful ways have provided a variety of fractionation methods designed to separate SOM into functional pools. Classical fractionation procedures separate SOM into humic acid, fulvic acid, and humin components as a function of relative solubility in strong acid and base solutions (Sollins et al. 1999). The humic acid component is soluble in base but not acid, the fulvic acid component is soluble in both base and acid, and the humin component is insoluble in base. However, few of these fractions have been shown to be ecologically relevant across widely different ecosystems, soil types, and management or disturbance regimes (Duxbury et al. 1990; Collins et al. 1999; Sollins et al. 1999). More useful are two simple chemical fractionations: water-soluble C extracts, which appear related to carbon readily available to microbes, and C released by acid hydrolysis. Acid hydrolysis appears to provide a reasonable measure of the resistant C pool in many soils; Leavitt et al. (1997) found for a variety of soils that nonhydrolyzable C is on average 1400 years older than the total C.

Physical fractionation methods use wet sieving, density flotation gradients, or chemical dispersal to separate SOM into pools of different size and stability classes. Recent work in the U.S. Great Plains (Cambardella and Elliott 1993) underscores the potential importance of soil aggregate formation for protecting SOM. Earlier work in a wide variety of soils (e.g., Ladd and Amato 1980; Spycher et al. 1983; Baldock et al. 1990; Christensen 1992) has shown that SOM particles of different densities decompose at different rates. Light fractions are typically comprised of partially decomposed plant residues with high decomposition rates (Gregorich and Janzen 1996). That aggregate formation and the distribution of SOM density classes are under largely biological control and subject to disturbances imposed by management practices (Oades 1993; Gregorich and Janzen 1996) suggests ecological relevance for these measures.

The proportional composition for any particular soil appears to be best quantified via microbial bioassays in which CO_2 release is followed over long-term incubations of a year or more. This biological fractionation relies on the in situ microbial and microarthropod community to define ecologically relevant SOM fractions. Biological fractionation schemes have arisen concurrent with the development of quantitative soil C models over the past two decades. These models have resulted from efforts to predict the loss rate and the eventual level of soil C following disturbance, and most now incorporate the concept of multiple soil C pools with different turnover times (Jenkinson et al. 1987; Parton et al. 1987; Paustian et al. 1992). Usually these models recognize at least three SOM pools (Fig. 7.1): an active fraction that turns over on the order of months to years, comprised of recent residues and microbial biomass and metabolites; a slow fraction that turns over on the order of decades, comprised of stabilized decomposition products; and a resistant fraction of highly stabilized, recalcitrant organic matter that turns over on the order of centuries to millennia.

The curves describing CO_2 release during long-term incubations can be parameterized into two or more components related to their proportional composition, as described later. These components have intrinsic ecological relevance, and a further value for their application in soil C models (e.g., see Fig. 7.1). The application of these models is becoming increasingly important for evaluating historical and future global change scenarios (e.g.,

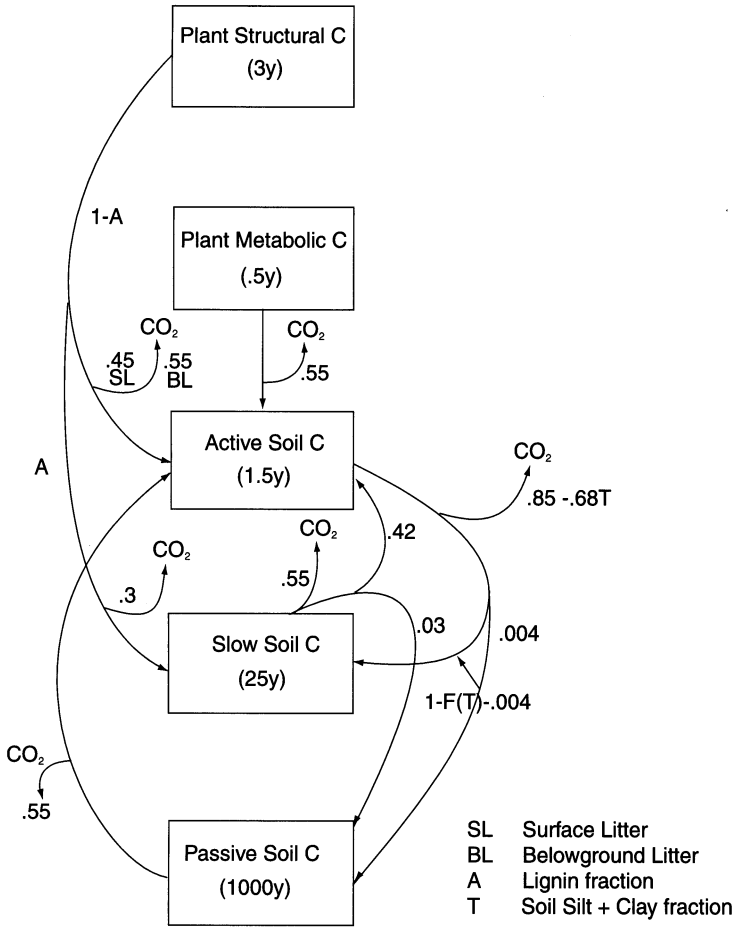

FIGURE 7.1. Structural components of the CENTURY soil organic matter model. Values adjacent to arrows are transfer coefficients for the noted carbon pools; parenthetical values are approximate turnover times. (From Paustian et al. [1992].)

Schlesinger 1984; Jenkinson et al. 1991; IPCC 1996).

In the remainder of this chapter we present and discuss appropriate methods for assessing plant litter decomposition and organic matter dynamics in terrestrial ecosystems. Dozens of contributions in the past decade have presented methods for assessing one or the other of these processes. We concentrate on those methods that have ecological relevance and that appear to be sufficiently well standardized to be usable for cross-ecosystem comparisons. This latter criterion is becoming increasingly important as the diversity of available methods limits opportunities for a posteriori syntheses of independently collected data from multiple sites and studies. Our contribution is intended to complement rather than complicate emerging efforts to standardize current soil methods for ecological research (e.g., Robertson et al. 1999b).

Plant Litter Decomposition

Fine Litter Decomposition Rates

The decomposition of plant leaves and other relatively fine material is typically assayed by following mass and nutrient changes in litter placed in mesh bags on the soil surface. As the organic material decomposes, changes in mass document the decomposition rate, and changes in nutrient composition document rates of nutrient release, retention, and immobilization (e.g., Falconer et al. 1933; Bocock et al. 1960; Gosz et al. 1973; Melillo et al. 1982; Heal et al. 1997).

Decomposition is carried out by a complex array of heterotrophic organisms that vary widely in size and function. Commonly these organisms are grouped into size (body-width) classes that correspond to the groups microflora and -fauna, and

macro-, meso-, and megafauna. The microheterotrophs are <100 μm and include mainly bacteria, fungi, nematodes, protozoans, and rotifers; the mesofauna are generally <2 mm in width and include mites, springtails, diplurans, and enchytraeids; and the macro-fauna include earthworms, isopods, spiders, molluscs and other organisms >2 mm width. Many of these organisms are detritovores, some are carnivores, and all appear to be important parts of the soil food web (Swift 1979; Beare et al. 1995; Coleman and Crossley 1996).

Investigations of decomposition with litter bags have employed different mesh sizes, sometimes to experimentally exclude different groups in order to determine their functional significance for decomposition (e.g., Heath et al. 1964). Generally, however, mesh size has been chosen to optimize the access of all organisms to entrained litter while minimizing excessive particle loss in situ. Particle loss to mineral soil is an important part of decomposition, and too small a mesh size will not only exclude certain organisms but will also inhibit particle loss. Mesh size may also affect litter microclimate (Witkamp and Olson 1963) if it inhibits air flow and water flux, and can also alter root ingrowth.

Although litter bag studies are compromised somewhat by mesh size limitations, alternatives to litter bags suffer from equal or greater problems. For example, litter baskets with mesh bottoms and mostly-open tops (Stevenson and Dindal 1981; Blair et al. 1991) allow greater access for macroinvertebrates and better represent the soil surface microclimate, but also allow additional litterfall inputs. Tethered material (Witkamp and Olson 1963; Lang 1974) is completely exposed to the biotic and abiotic soil surface environment but even large pieces that fragment from the original material can be difficult to recover for later weighing and chemical analysis. Thus, litter bags with an appropriate mesh size remain the preferred means for directly estimating fine litter mass loss (Harmon et al. 1999).

Indirect measures of estimating decomposition include estimates of the decomposition rate constant k using litter input: forest floor ratios (Olson 1963), and annual differences in the lowest and highest stores of litter (e.g., Loomis 1975). These methods are usually less preferable than direct means because of their underlying assumption of steady state conditions and the requirement for precise measurements of litter stores, especially where decomposition rates are low. In some environments, most notably annual cropping systems, these indirect techniques to estimate mass loss can be quite accurate.

To allow access to macrofauna, litter bag mesh size must be >2 mm although 1-mm nylon mesh has been used in most recent litter bag studies. Harmon et al. (1999) recommend a 1.5-mm fiberglass mesh for high-light environments in which nylon and other organic materials will be subject to ultraviolet (UV) attack. This is probably a reasonable compromise that allows access for most mesofauna, that preserves a near-normal litter layer microclimate, and that avoids excessive loss of large litter fragments. No mesh size will be free of compromising assumptions, however, and results from any litter bag study must be interpreted accordingly. Coleman et al. (1999) recommend specific procedures for assessing macroinvertebrate contributions to decomposition rates, and these should be consulted for ecosystems in which macroinvertebrates will substantially affect decay rates.

The size of the litter bag must also be appropriate to the litter under investigation; 20 × 20 cm is conventional, but bags may need to be larger for larger litter or for litter from more diverse plant communities. Depending on the purpose of the study, the contents of each litter bag should be proportionately representative of the target ecosystem: single versus mixed-species litter bags are known to exhibit different decomposition dynamics (Blair et al. 1990), and in many plant communities it will be appropriate to include fine woody debris (e.g., twigs). Litter should be freshly senesced.

The number of litter bags to deploy at a site will vary as a function of expected decomposition rate and the microclimatic heterogeneity of the site. The minimum number of bags must be sufficient to describe the decay curve with enough confidence that the decomposition rate constant k can be adequately estimated. Usually a geometric sampling interval is appropriate, that is, more bags are retrieved from the field earlier than later in the study. Often 3 to 6 collections arranged by season are adequate for the first year of the study and 2 to 4 collections in subsequent years. Most studies have examined decomposition dynamics over 1- to 3-year periods; consequently we know less about late-term decom-

position in most environments, so there is, in fact, considerable value for extending studies to >5 years (Harmon et al. 1999).

At least 4 to 5 litter bags should be retrieved at each sampling interval for each experimental or landscape replicate, which should be chosen to avoid pseudoreplication. Thus, it is not uncommon to deploy 100 or more litter bags to appropriately characterize decomposition within an ecosystem. For postcollection chemical analyses it may be appropriate to composite samples after drying and weighing. Samples to be chemically analyzed should be dried at 55°C to avoid heat-induced chemical changes in C content. Both litter mass and sometimes chemical content must be corrected for contaminating soil that is commonly collected with the litter bags, especially at later stages of decomposition. Mass can be corrected by combusting each sample and calculating an ash-free dry mass proportion to estimate the litter versus soil portion of the sample. Where contaminating soil has a high organic matter content or can affect nutrient analyses, a more involved correction is necessary (Blair 1988; Harmon et al. 1999).

The decomposition rate constant k can usually be calculated by fitting mass loss to the single negative exponential model (Jenny et al. 1949; Olson 1963):

$$x_t/x_o = e^{-kt} \quad (7.1)$$

where x_t/x_o is the proportion of original mass remaining at time t, t is elapsed time in years, and k is the decomposition rate constant.

Least-squares regression (e.g., Fig. 7.2) will provide values for k, for the y-intercept, and confidence intervals. The y-intercept can be used to indicate the presence of initial decomposition stages that are more accelerated or slower than that predicted by the model (Harmon et al. 1999). In these cases, alternative models may be more appropriate.

A wide variety of chemical analyses have been applied to decomposing litter in attempts to identify functional predictors of decay and nutrient mineralization rates (see Heal et al. 1996 for a recent review). Recent efforts have identified total polyphenol content and protein-binding capacities of litter as useful predictors of rates (Palm and Sanchez 1991; Handayanto et al. 1997) in addition to the more conventional nitrogen and lignin contents

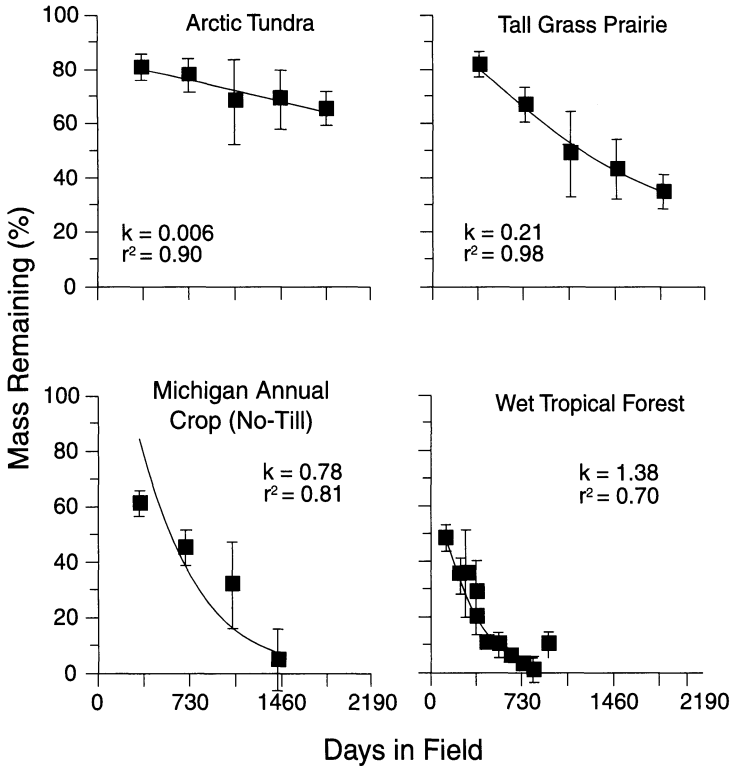

FIGURE 7.2. Mass loss from litter bags containing wheat litter from a southern Michigan agricultural site placed in four different ecosystems. The decomposition rate constant k is calculated by fitting the exponential decay equation noted in text and diagrammed in each graph. (From Halstead et al. [1996].)

(e.g., Melillo et al. 1982; Aber et al. 1990). Palm and Rowland (1997) suggest a standard set of analyses for characterizing plant litter quality, as do Harmon and Lajtha (1999).

Woody Detritus

Woody detritus includes both fine and coarse woody debris. Fine fractions are defined operationally as dead trees and branches <10 cm in diameter and <1 m long, as well as twigs and roots too large for litter bags. Coarse fractions include boles, branches, and roots larger than fine fractions. The principal reason for dividing woody detritus into these fractions is mode of study: fine fractions can be weighed intact while coarse fractions must be subsampled in the field.

Methods for studying woody detritus are analogous to litter bag methods but usually without the need for litter confinement. Woody material can be cut to standard lengths (a typical length is 10 times longer than the mean diameter of the material), tethered and marked for later collection, and placed in the field in microclimates that would be normally encountered—on the litter layer, suspended above the soil surface (for standing dead material such as dead branches still attached to stems), or buried (for coarse roots).

At intervals appropriate to the climatic environment and composition of the wood, tethered pieces are collected and taken to the laboratory for drying, weighing, and chemical analysis. A minimum of 10 sample collections will likely be needed, spaced at 1- to 3-year intervals. Any given study may take decades to complete.

Decomposition of woody material is a function of size and species as well as placement, so the number of samples initially deployed will need to be chosen carefully to provide a geometric range of diameter classes and an appropriate range of genera. It is usually not necessary to sample below the genus level except for genera with diverse heartwood contents. Because bark, sapwood, and heartwood decay at different rates, it is important to record the proportions of these materials in the pieces initially deployed. As for fine litter, replication should be organized to avoid pseudoreplication. Sample preparation, chemical analyses, and calculations are similar to those for fine plant litter, though analyses should be performed separately for bark, sapwood, and heartwood.

Because it is impractical to weigh large woody debris, one can remove trimmed end pieces from the field for weighing and chemical analyses. Prior to initial sampling, it is necessary to estimate the mass of each piece that will eventually be removed; this should be done at the outset of the study by measuring in situ the volume of each segment that will be removed and assuming an initial density ($g\ cm^{-3}$) equal to the density of the first sample. Formulas for determining the volume of complex shapes are provided in Harmon et al. (1999).

Reciprocal Transplants and Standard Substrates

The relative influence of climate versus litter quality for controlling decomposition rates can be evaluated using reciprocal litter transplants and standard substrates. Reciprocal litter transplants usually involve exchanging litter bags or tethered wood among two or more sites: litter from ecosystem A is allowed to decompose in both ecosystem A and ecosystem B, as is litter from ecosystem B. This scheme effectively removes litter quality as an explanation for differences in the decomposition rates of litter from the two ecosystems. For example, ecosystem A litter should decompose at the same rate in each ecosystem if the decomposition environment of ecosystem A is similar to that of ecosystem B; a faster rate of litter disappearance in ecosystem B means a more favorable environment in ecosystem B (see Fig. 7.2).

Different rates of decay are usually interpreted to mean different abiotic conditions among sites, that is, between-site differences in moisture, temperature, and (for buried litter) aeration regimes. However, differences in the biotic environment can also lead to different decomposition rates among ecosystems, and these differences need also to be kept in mind when interpreting transplant results. For example, differences in the soil invertebrate community and, perhaps to a lesser extent, differences in the microbial community may accelerate decomposition in one ecosystem relative to another; separating abiotic from biotic factors requires a more elaborate experimental design. Notwithstanding the difficulty of separating the effects of the abiotic from the biotic environment, recip-

rocal transplants can provide a valuable approach for evaluating differences in substrate quality as an explanation for decomposition rate differences among divergent ecosystems.

Substituting a standard substrate for native litter provides a similar and logistically simple means for evaluating substrate quality versus environment differences. In this technique (e.g., Binkley 1984; Sinsabaugh et al. 1993; O'Lear et al. 1996) a single organic substrate, usually one that is manufactured and thus commercially available, is placed in litter bags or buried across a range of two or more ecosystems. Studies have employed cellulose filter paper and hardwood dowels as substrate standards. Both filter paper and dowels vary somewhat in their substrate composition, however—filter paper can contain small but varying amounts of nitrogen, and dowels can contain different amounts of decay-resistant heartwood—so it is necessary to select materials carefully. Because of substrate differences, results may still be interpretable only within the scope of a particular study. Harmon et al. (1999) recommend dowels be made of basswood (*Tilia* spp.), birch (*Betula* spp.), or ramin (*Gonystylus bancannus*), 6 mm in diameter by 60 cm in length. Placement, recovery, and analysis of standardized substrates follow the same procedures as for fine and coarse woody litter, above.

Soil Organic Matter Dynamics

Soil Organic Matter Stores

Soil organic matter is defined operationally as C and associated nutrients entrained in soil mineral horizons, in forms no longer easily identifiable as plant residues. The O horizon or litter layer is by convention excluded from SOM stores, because it can be identified as specific plant residues that in modeling are handled by separate equations. In most soils, the mass of C in mineral horizons far outweighs the mass of C in the litter horizon. Soil, however, includes the entire solum, and especially in ecosystem studies it is important to include C in horizons both above and below the more commonly analyzed A horizon when calculating total ecosystem SOM stores. This may mean sampling to a meter or more in many soils. Sites in which significant decomposition occurs in the tree or grass canopy present a special challenge, as this material also is part of ecosystem decomposition.

Litter stores are easily evaluated by collecting litter from known-area quadrants (e.g., 1 m^2) and analyzing for C and nutrients as for leaf and woody material, above. In most ecosystems, stores will vary by season and collections should be staggered accordingly. Stores of organic C in mineral soil are usually evaluated by whole-soil combustion. Any inorganic C that may be present is either removed beforehand or analyzed separately and subtracted from combustion results. Inorganic C can be a major fraction of total C in calcareous soils low in SOM; this is often the case for soils in arid and semiarid environments, for young soils developed on carbonate parent materials, and for agricultural soils limed for pH control.

Modern C and N (CN) analyzers oxidize small samples at high temperatures (>1000°C) in an O_2-enriched atmosphere, then measure the resulting gases by gas chromatography (CO_2 and N gases) or infrared gas analysis (CO_2 only) (Nelson and Sommers 1996). Small sample size (usually <20 mg) means that great care must be taken to adequately subsample and powder the soil to be analyzed; inadequate grinding is the leading source of analytical variability for most soils (Sollins et al. 1999). Conversion of gravimetric results (e.g., g C/g $soil^{-1}$) to an areal extent (e.g., g C m^{-2}) is crucial for including SOM C in total ecosystem carbon budgets and for making comparisons of SOM across ecosystems with different soil types and depths. It is thus critically important to have accurate values for both soil bulk density (g soil cm^{-3}), stone content, and soil horizon depths. Bulk density values are needed to convert from gravimetric values to areal values, stone volume must be excluded from the conversion, and estimates must be weighted by horizon depths, including the depth of the O or litter layer horizon (Sollins et al. 1999). Alternately, single soil cores taken to a depth of minimal soil C content can be homogenized and analyzed for total C content, and this total-core value then divided by the sampled stone-free surface area to yield an areal estimate for SOM C.

Physical Fractionation of Soil Organic Matter

The two most promising strategies for physically separating SOM into differentially reactive fractions are fractionations based on density and size

of both the plant residues and soil aggregates. Soils are typically comprised of a wide variety of particles that differ in size, shape, mineral composition, and density. Typically, these particles exhibit associated differences in C content and the availability of this C to microbial oxidation. Ecosystem-level differences in these properties can provide insight into differences among ecosystems and disturbance regimes with respect to SOM dynamics.

Density fractionation is useful where lighter particles exhibit different C dynamics than heavier particles; most obvious are density differences between organic materials, most of which are light, and mineral-associated materials, most of which are heavy (Gregorich and Janzen, 1996). At intermediate densities are a wide range of materials, including amorphous, mineral material adsorbed onto organic fractions, secondary minerals such as clays coated with different thicknesses of organic compounds, and soil aggregates of differential stabilities (Turchenek and Oades 1978; Spycher et al. 1983; Tisdall 1996; Sollins et al. 1999). The type of mineral present can greatly affect decomposition rates, especially when clays with mixed exchange characteristics are involved (Sollins et al. 1999).

Soils are most easily separated into density fractions using flotation. The simplest method is to mix the soil with an inert heavy liquid such as sodium iodide (NaI) or sodium polytungstate (NaPT) and remove the floating fraction (Strickland and Sollins 1987). By sequentially adjusting the density of the NaI or NaPT, soil fractions of differing densities can be collected sequentially, usually across an initial range of 1.0 to 2.0 g cm^{-3}. Initial suspensions can be mixed or sonicated to disperse aggregates of different stabilities. Each fraction can then be analyzed for C and N content. Sollins et al. (1999) provide a specific procedure.

Size fractionation (as opposed to density fractionation) is based on the fact that different particle sizes are differentially reactive because of differences in specific surface areas. For example, clay-size particles (<2 μm in diameter) have a surface area that is 10^4 larger than the surface area of sand-size particles (<2 mm in diameter): approximately 10^2 versus 10^6 $cm^2/g\ soil^{-1}$, respectively. One might thus expect the smaller particles to have adsorbed a greater fraction of organic material that will also be more accessible (because of its larger surface area) to microbial oxidation.

A number of techniques are available for sorting soil into size classes for subsequent chemical analysis (Gee and Bauder 1986; Elliott et al. 1999). Efforts to correlate SOM size class fractions with ecosystem attributes have successfully used within-aggregate particulate organic matter (Cambardella and Elliott, 1992, 1993) and light fraction (Gregorich and Janzen 1996) components. These coarse particulate organic matter fractions appear to correlate well with land use management and vegetation changes and with differences in CO_2 evolution in extended laboratory incubations. Carter and Stewart (1996) review the relationship between soil structure, various soil C fractions, and SOM storage and turnover.

Biological Soil Organic Matter Fractions

Biological fractions are defined by the relative rates of SOM turnover in short- versus long-term soil incubations. Rates of CO_2 release during incubations typically follow an exponential decay curve, that is, CO_2 release is much faster earlier in the incubation than later. As the more labile C is oxidized in the incubation, the slow-turnover material is unmasked and both fractions can then be compared with the total C pool to derive fast- and slow-turnover SOM fractions (Fig. 7.3). The turnover of the old resistant pool can be determined by acid hydrolysis (and if necessary confirmed by C dating) and is expressed as mean residence time. The amount of C coming from the old pool is small in laboratory incubations. The mean residence time therefore has little effect on the determination of the dynamics of the active and slow pools, although the size of the old pool enters into the calculations and should be estimated independently by acid hydrolysis.

Laboratory-derived decomposition rate constants can be converted to mean residence times by the knowledge that in first order reactions at steady state, mean residence time is $1/k$. The laboratory mean residence times can be converted to field mean residence times through the use of Q_{10} relationships and knowledge of the field mean annual temperatures. As noted above, the resistant pool is measured with acid hydrolysis (Martel and Paul 1974, Leavitt et al. 1997). These three pools analytically define the conceptual pools of the same names that are incorporated in leading SOM mod-

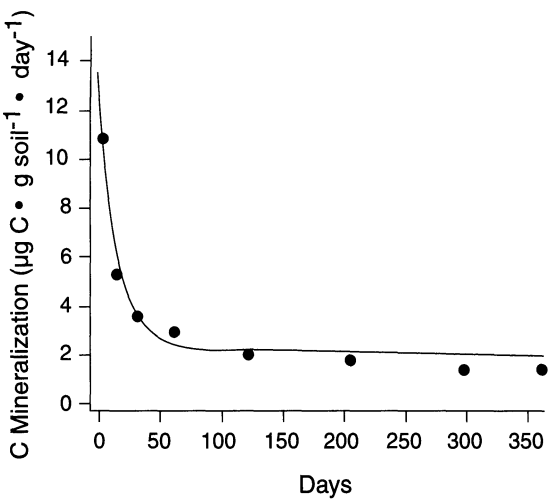

FIGURE 7.3. Long-term carbon mineralization in a cultivated soil from an agricultural site in southwest Michigan. The solid line is fit with the 2-pool nonlinear model noted in text. The model provides estimates of the fast and slow turnover pools; the passive or resistant pool can be estimated by acid hydrolysis. (From Paul et al. [1999].)

els (see Fig. 7.1) (Paul et al. 1999, in press). Models make it possible to correct for the effects of microbial growth and the knowledge that materials in soil move from one pool to the other. Analytically determined SOM decomposition kinetics greatly assist model improvement and in turn benefits our empirical understanding and application of SOM knowledge (Paustian et al. 1992; Paul et al. 1997)

A variety of short-term incubation techniques have been developed that involve measuring CO_2 release from incubated soil over 1- to 24-hour incubation periods. This release provides a quick snapshot of the quantity of readily mineralizable C in one soil versus another. Methods range from simple incubations of intact soil cores brought to some optimal proportion of water-holding capacity in the laboratory (e.g., Robertson et al. 1988; Collins et al. 1999) to more elaborate shaken-slurry incubations that—when accompanied by a glucose-amended companion slurry—can provide a relative measure of microbial biomass (e.g., Anderson and Domsch 1989; Beare et al. 1990).

In many soils, an initial CO_2 flush associated with soil sampling, mixing, and sieving can distort estimates of the size of the fast turnover pool, so some methods—especially long-term incubations—employ a preincubation interval of several days. Preincubation is not important for within-site comparisons of immediately available SOM C or for intact-core samplings, but for cross-study comparisons in which samples may be dried, differentially mixed, sieved, or brought to a new moisture content, preincubation can be important to normalize for differential sampling effects and for differences in rainfall and temperature patterns in the days immediately prior to sampling.

Robertson et al. (1999a) recommend a technique in which unamended soil is brought to the laboratory and, after sieving, is incubated at optimal water content (approximately 60% water-holding capacity) in an aerobic atmosphere for 7 days. At the end of 7 days, the soil's respiration potential is assessed by measuring the rate of CO_2 release over a 2 to 3-hour period during which soil head-space is sealed from the atmosphere. Carbon dioxide is readily measured by introducing 1-ml samples into a stream of N_2 carrier gas connected to an infrared gas analyzer. This technique can be amended to follow long-term CO_2 release by repeating CO_2 analyses at geometrically increasing intervals (e.g., 14, 28, 42, 63, 84, 105, 140, 196, and 252 days). Long-term N release can be followed concomitantly by sacrificing subsamples at each interval for inorganic N analysis.

Long-term incubations can be used to estimate multiple SOM pools by fitting the following model to a graph of CO_2 production (e.g., CO_2-C g^{-1} day^{-1} or CO_2-C cm^{-2} day^{-1}) versus incubation time:

$$\text{C Mineralization}_t = k_1(C_1e^{-k_1t}) + k_2(C_2e^{-k_2t}) \quad (7.2)$$

where C_1 is carbon content of the active or fast turnover pool, k_1 is the rate constant for the C_1 pool, C_2 is carbon content of the intermediate or slow turnover pool, k_2 is the rate constant for the intermediate pool, and t is incubation time in days.

The third, resistant pool can be very roughly estimated as total C (SOM stores, above) less C_1 and C_2. A more accurate estimate of the resistant pool can be made by using acid hydrolysis to define its size and by using the ^{14}C age of the hydrolysis residue to estimate the rate constant for this pool (Paul et al. 1998). Because of the great age of this pool, an average age of 500 y can be assumed or one can

use a two-pool constrained model once the size of the resistant pool is known (Paul et al. 2000).

Statistical analysis of the curves is most easily accomplished when the CO_2 evolution data are expressed on a rate per unit time basis rather than as cumulative curves (Hess and Schmidt 1995). Use of all the data in a single regression equation with separate parameter estimates for each treatment provides much greater statistical power than analyzing each treatment in a separate regression (Willson et al. in press). The effect of possible autocorrelation from repeated measurements of the same sample also can be statistically corrected (Paul et al. in press).

Use of Tracers

The isotopes of C include the radioactive ^{14}C occurring in minute quantities in the atmosphere and ^{13}C, which has a stable natural abundance of 1.1%. The $^{14}CO_2$ produced in the atmosphere by the interaction of cosmic radiation with N_2 is incorporated into plants during photosynthesis at a fairly constant rate, and can be measured with carbon dating techniques (Goh 1991). This is expensive and not readily accessible, but together with acid hydrolysis gives the best estimates of the size and turnover of the old resistant SOM fraction (Paul et al. 1998; Leavitt et al. 1997). Added ^{14}C, in the form of uniformly labeled plant residues exposed to a high ^{14}C atmosphere, give exceptionally useful information on organic matter breakdown and the incorporation of C into microbial biomass (Wolf et al. 1994). It is difficult to produce uniformly labeled substrates, but field experiments can be undertaken with commercially available, specifically labeled substrates if adequate health and safety precautions for working with a radioactive isotope are followed (Schimel 1993).

The stable isotope ^{13}C has been utilized in experiments in which plants have been ^{13}C-labeled in the laboratory in a fashion similar to that used for radioactive elements. Reliable automated mass spectrometers to measure the stable isotopes are available in many laboratories. The naturally occurring discrimination against $^{13}CO_2$ by plants with a C_3 photosynthetic pathway relative to those with a C_4 pathway provides a powerful, naturally occurring label in many parts of the world. This requires a C_3–C_4 plant growth switch, such as the growth of C_4 corn or sorghum on sites previously forested with C_3 trees, or the growth of C_3 wheat, rice, cold season grasses, or most trees on prairie or savanna soils that previously had C_4 native vegetation. Sites that have a mixed signal make it possible to grow either a C_3 or C_4 test species. The ^{13}C either enriched or depleted in the test species can be used to measure the fate of test species plant residues—both their incorporation into various soil biological, chemical, and physical fractions as well as changes in their pool sizes and flux rates.

^{13}C studies are particularly useful when combined with CO_2 evolution studies (Paul et al. 1999). The measurement of the $^{13}CO_2$ evolved during extended laboratory incubation yields estimates of the active and slow pool sizes and decomposition kinetics for the labeled material. Details of these techniques are available in Schimel (1993), Boutton (1996), Coleman and Fry (1991), and Paul et al. (1998, 1999). The element N is closely associated with soil C, and the stable isotope ^{15}N has been utilized in many residue decomposition and SOM turnover studies. It, too, can provide much important information on decomposition as well as on the microbial growth and N immobilization that occurs during decomposition (Hauck et al. 1994).

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